

CLAIMS

1. (Amended) A production method of a mammalian artificial chromosome, comprising:

- 5 a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into a mammalian host cell;
- a second step of selecting transformed cells; and
- 10 a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells.

2. (Amended) A production method of a mammalian artificial chromosome, comprising:

- 15 a first step of introducing a first vector consisting of a yeast artificial chromosome having a mammalian centromere sequence and a mammalian telomere sequence and a second vector consisting of a yeast artificial chromosome having an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into a mammalian host cell;
- 20 a second step of selecting transformed cells; and
- a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells.

3. The production method according to claim 1 or 2, wherein the first vector
25 has a selection marker gene and the selection of the transformed cells in the second step is carried out by using the selection marker gene.

4. The production method according to any of claims 1 to 3, wherein the mammalian centromere sequence comprises a region in which a plurality of the
30 following sequences are arranged at regular intervals:

5'-NTTCGNNNNANNCGGGN-3': SEQ ID NO. 1, wherein N is selected from the group consisting of A, T, C and G.

5. The production method according to any of claims 1 to 4, wherein the mammalian centromere sequence comprises a sequence derived from a human chromosome alpha satellite region.

6. The production method according to claim 5, wherein the mammalian centromere sequence comprises a 11mer repeat unit derived from a human chromosome 21.

7. The production method according to any of claims 1 to 6, wherein the size of the mammalian centromere sequence is about 50 kb or less.

15 8. (Cancelled)

9. (Cancelled)

10. (Cancelled)

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11. (Cancelled)

12. (Cancelled)

25 13. (Amended) The production method according to any of claims 1 to 7, wherein the insertion sequence is a loxP site, a FRT site, or a sequence obtained by partial modification of a loxP site or a FRT site and has a function for inserting the sequence of interest.

30 14. The production method according to any of claims 1 to 13, wherein the

quantity ratio of the first vector to the second vector, which are inserted in the first step, is in the range from about 10 : 1 molecular ratio to about 1 : 10 molecular ratio.

5 15. (Cancelled)

16. (Cancelled)

10 17. (Amended) A mammalian artificial chromosome obtainable by the production method described in any of claims 1 to 16,

which comprises a mammalian replication origin, a mammalian centromere sequence, an insertion sequence for specifically inserting a sequence of interest and an insulator sequence; and

15 which is circular in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.

18. (Amended) A mammalian artificial chromosome obtainable by the production method described in any of claims 1 to 16,

20 which comprises a mammalian replication origin, a mammalian centromere sequence, a mammalian telomere sequence, an insertion sequence for specifically inserting a sequence of interest and an insulator sequence; and

25 which is linear in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.

19. (Cancelled)

20. (Cancelled)

21. (Cancelled)

22. (Cancelled)

5 23. (Amended) A mammalian artificial chromosome,

which comprises a mammalian replication origin, a mammalian centromere sequence, an insertion sequence for specifically inserting a sequence of interest and an insulator sequence, and

10 which is circular in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.

24. (Amended) A mammalian artificial chromosome,

which comprises a mammalian replication origin, a mammalian centromere sequence, a mammalian telomere sequence, an insertion sequence for specifically inserting a sequence of interest and an insulator sequence,

which is linear in form and is replicated in a mammalian cell, maintained extrachromosomally in a host cell, and transmitted to daughter cells during cell division.

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25. The mammalian artificial chromosome according to claim 23 or 24, wherein the insertion sequence is a loxP site, a FRT site, or a sequence obtained by partial modification of a loxP site or a FRT site and has a function for inserting the sequence of interest.

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26. The mammalian artificial chromosome according to any of claims 17 to 25, wherein the mammalian centromere sequence comprises a region in which a plurality of the following sequences are arranged at regular intervals:

30 5'-NTTCGNNNNANNCGGGN-3': SEQ ID NO. 1, wherein N is selected from the group consisting of A, T, C and G.

27. The mammalian artificial chromosome according to any of claims 17 to 25, wherein the mammalian centromere sequence comprises a sequence derived from a human chromosome alpha satellite region.
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28. The mammalian artificial chromosome according to claim 27, wherein the mammalian centromere sequence comprises an 11mer repeat unit derived from a human chromosome 21.
- 10 29. The mammalian artificial chromosome according to any of claims 17 to 28, comprising a plurality of the functional sequences or the insertion sequences.
30. (Cancelled)
- 15 31. A mammalian cell containing the mammalian artificial chromosome described in any of claims 17 to 30 outside the autonomous chromosome.
32. A human cell containing the mammalian artificial chromosome described in any of claims 17 to 30 outside the autonomous chromosome.
- 20 33. An embryonic stem cell containing the mammalian artificial chromosome described in any of claims 17 to 30 outside the autonomous chromosome.
- 25 34. A production method of a mammalian cell in which the functional sequence or the insertion sequence is introduced in a state in which they can be maintained stably for a long term, the method comprising:
introducing the mammalian artificial chromosome obtained by the production method described in any of claims 1 to 16 or the mammalian artificial chromosome described in any of claims 17 to 30 into mammalian cells as target
- 30

cells.

35. (Amended) A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

- 5 a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into mammalian host cells;
- 10 a second step of selecting transformed cells;
- 10 a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- 10 a fourth step of isolating the mammalian artificial chromosome from the selected cells; and
- 15 a fifth step of introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

36. (Amended) A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

- 20 a first step of introducing a first vector consisting of a yeast artificial chromosome having a mammalian centromere sequence and a mammalian telomere sequence and a second vector consisting of a yeast artificial chromosome having an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into mammalian host cells;
- 25 a second step of selecting transformed cells;
- 25 a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- 25 a fourth step of isolating the mammalian artificial chromosome from the selected cell; and
- 30 a fifth step of introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

37. (Amended) A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:

- a first step of introducing a first vector being circular in form and comprising a mammalian centromere sequence and a second vector being circular in form and comprising an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into mammalian host cells;
- 5 a second step of selecting transformed cells;
- a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- 10 a fourth step of fusing the selected cell with a mammalian cell having an ability of forming micro-cells;
- a fifth step of selecting a hybrid cell capable of forming micro-cells and containing the mammalian artificial chromosome; and
- 15 a sixth step of forming micro-cells from the selected hybrid cell.

38. (Amended) A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:

- a first step of introducing a first vector consisting of a yeast artificial chromosome including a mammalian centromere sequence and a mammalian telomere sequence and a second vector consisting of a yeast artificial chromosome including an insertion sequence for specifically inserting a sequence of interest and an insulator sequence into mammalian host cells;
- 20 a second step of selecting transformed cells;
- 25 a third step of selecting a cell containing a mammalian artificial chromosome from the selected transformed cells;
- a fourth step of fusing the selected cell with a mammalian cell having an ability of forming micro-cells;
- a fifth step of selecting a hybrid cell having an ability of forming
- 30 micro-cells and containing a mammalian artificial chromosome; and

a sixth step of forming micro-cells from the selected hybrid cell.

39. A production method of mammalian cells containing a mammalian artificial chromosome, comprising:

5 fusing the micro-cell obtainable by the production method described in claim 37 or 38 with a mammalian cell as a target cell.

40. A production method of a mammalian cell containing a mammalian artificial chromosome, comprising:

10 isolating the mammalian artificial chromosome from the host cell containing the mammalian artificial chromosome described in any of claims 17 to 30; and

introducing the isolated mammalian artificial chromosome into a mammalian cell as a target cell.

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41. A production method of a micro-cell containing a mammalian artificial chromosome, the method comprising:

20 fusing a host cell containing the mammalian artificial chromosome described in any of claims 17 to 30 and a mammalian cell having an ability of forming micro-cells;

selecting a hybrid cell having an ability of forming micro-cells and containing the mammalian artificial chromosome; and

forming micro-cells from the selected hybrid cells.

25 42. A production method of a mammalian cell containing a mammalian artificial chromosome, the method comprising:

fusing the micro-cell obtainable by the production method described in claim 41 with a mammalian cell as a target.

30 43. The production method of a mammalian cell according to any of claims

34, 35, 36, 39, 40 and 42, wherein the mammalian cell as a target cell is an embryonic stem cell, embryonic germ cell, or tissue stem cell.

44. The production method of a mammalian cell according to any of claims
5 34, 35, 36, 39, 40 and 42, wherein the mammalian cell as a target cell is formed by inducing an embryonic stem cell, embryonic germ cell, or tissue stem cell so as to be differentiated to a cell of specific tissue.

45. The production method of a mammalian cell according to any of claims
10 34, 35, 36, 39, 40 and 42, wherein the mammalian cell as a target cell is a fertilized egg.

46. (Cancelled)

15 47. (Cancelled)

48. (Cancelled)

49. (Cancelled)

20 50. A vector used for producing a mammalian artificial chromosome, comprising: a sequence of a loxP site or FRT site, or a sequence obtainable by partial modification of a loxP site or FRT site, the sequence having a function for inserting the sequence of interest, and
25 an insulator sequence.

51. (Cancelled)

52. (Amended) A non-human transformed animal into which the mammalian
30 artificial chromosome described in any of claims 17 to 19 is introduced.

53. (Cancelled)

54. (Amended) An XO type mouse embryonic stem into which a mammalian
5 artificial chromosome described in any of claims 17 to 19 is introduced

55.(Cancelled)

56. (Amended) A female chimeric mouse into which a mammalian artificial
10 chromosome described in any of claims 17 to 19 is introduced.